pression strains which take place in the synclinal fold of the Cumberland. It is readily to be perceived that the nature of the strains developed by the synclinal folds must vary greatly from those which are formed beneath the anticlinals of a mountain district.

I only propose to call attention to the great problems in structural geology which this region presents to us, with a view of interesting our students of dynamic geology in their solution. More extended discussions of these questions will be given in the forthcoming volumes of the Memoirs of the Kentucky Geological Survey.

THE STUDY OF ZOÖLOGY IN GERMANY.

BY CHARLES SEDGWICK MINOT.

II. THE METHODS USED IN HISTOLOGY AND EMBRYOLOGY.

THE use of the microscope goes hand in hand with the work of zoölogists in Germany, and it is there that we find the greatest number of means employed to render the objects suitable for examination. I have frequently heard American zoölogists express a slight distrust of histological methods, — well founded, perhaps; it ought not to lead to the rejection of the benefits to be obtained from using them, but merely to greater caution in employing them.

It is well known that animal tissues and organs consist of cells of various kinds, variously grouped together. The forms which these cells can assume lead to the most curious transformations, so that things as different from one another as muscular fibres, blood corpuscles, and ganglion cells can be traced as modifications of the same primitive form. The work of microscopic anatomists is to detect the changes which the simple cells of embryos undergo in the course of their transformations into the components of the tissues of the adult, and to investigate in detail the final results of these metamorphoses. It is much to be desired that America should assist more in this work, and it is with the hope of stimulating some persons to do so that this article is written.

In the tissues of the adult we find the cells arranged in a definite manner, and we have consequently to examine the shape and character of the single cells, and then their relation to one another. Simply placing a small piece of an organ underneath

the microscope is not sufficient to enable us to do this, but we are obliged in every case to subject the preparation to a special treatment. The first thing to be done is to make the object transparent enough to let the light pass through it to the objective, which is usually done by mounting it in glycerine or in Canada balsam, both of which substances have a high index of refraction, and therefore when they penetrate the interstices of a tissue do away with the refraction inside of it, so to speak; for in every tissue the different parts refract the light so variously that a ray passing through frequently changes its path, thus confusing the final image which reaches the observer's eye. A layer of powdered glass lets the light pass through, but nothing distinct can be seen; if, however, the whole is immersed in Canada balsam, it immediately becomes beautifully transparent, because the balsam fills up the spaces between the bits of glass, and since balsam and glass refract light to about the same degree the mass becomes optically nearly uniform, and a ray of light can pass through it without being deviated from its course or destroying the image. The action on the tissues is identical, — and this should be carefully remembered, because balsam renders objects more transparent than does glycerine, so that in some cases one liquid is better than the other. It is a sign of inexperience to assert that balsam is better than glycerine, or *vice versa*, for they are both useful, but for different purposes.

In order to observe the cells well it is necessary not to have too many superposed layers in the field of view, but to make the object as thin as possible. This is usually accomplished by making sections. So important and so useful are such very thin slices that probably nine tenths of every histological collection consist of them. The first thing, therefore, is to acquire skill in making sections, and the perfection reached will mainly decide how far the progress of the student shall continue. The importance and benefits of making sections have led to the invention of a great many mechanical contrivances for cutting them. One form of cutter or microtome well adapted to its object was described in the April number of the NATURALIST of this year. Numerous other forms have been suggested, but those with which I am acquainted all have some defects. Free-hand cutting still remains absolutely indispensable. It may be acquired by patient practice even by those who have no special manual skill, just as we are all able to write. There are many things which cannot be cut with a machine. The razor for cutting should be

of the best quality, and when used always drawn towards the body, while the surface, which looks downward in cutting, must be flat. The edge must be perfect, the slightest notch being sufficient to tear a section to pieces, and so sharp that a human hair can be split with it. The sections themselves must be as thin as possible.

Since all parts of the body, with few exceptions, such as the skeleton, etc., are soft and permeated by water, besides possessing great elasticity, they cannot be cut in their natural condition; it becomes necessary, therefore, to harden the organs. Now protoplasm is the main constituent of cells, and itself consists chiefly of albumen. This substance can be coagulated by the action of various agents, some of which can be applied to the tissues without injuring them, to produce a coagulation of the albumen in its natural form within the cells.

Alcohol is one of the most valuable agents for this use. It produces its effect by its strong affinity for water, which it can withdraw from the tissue, thus causing the albumen, which requires an abundance of water to maintain its semi-fluid state, to solidify. It may be employed for the majority of tissues with perfect success. The volume of alcohol should be from twenty to thirty times that of the object to be hardened; weaker alcohol, say of eighty per cent., should be used first; after a sojourn of an hour or two, or even longer, if large, the object may be transferred to stronger (ninety-six per cent.) spirit and there left for twenty-four hours, more or less, according to the size of the piece. The great difficulty in the use of alcohol is to prevent the shrinkage which naturally follows upon the abstraction of the water from the tissues. This may be avoided by using first weak, and then strong, and finally very strong spirit. In some cases the action is not even then sufficient, and recourse must be had to absolute alcohol, which generally produces the desired result.

When even that does not succeed the specimens may be put in picric acid (concentrated cold aqueous solution) for twenty-four hours, then in a syrupy solution of gum arabic for twenty-four hours, and finally in strong alcohol again for the same length of time. The picric acid removes the alcohol, and allows the gum to penetrate the object, within which it is finally coagulated by the last dose of spirit. The sections when made must be left in water for a day, to dissolve out the gum which they still contain, and which renders them quite opaque. A very few

drops of strong carbolic acid may be added to the water to prevent the development of bacteria, etc., which would quickly ruin the preparations. Coagulated gum renders the majority of organs of a pleasant consistency for cutting.

Instead of gum, paraffine may be made to permeate the tissues, in the way already described in detail in the article on the sledge microtome, in the April NATURALIST.

All acids produce in albumen chemical changes, which, without withdrawing the water, cause coagulation. There are some which are admirably suited for hardening agents. Foremost among these is chromic acid, first introduced by Hannover, in 1841, from motives of economy. It is employed in solutions of two fifth parts for one thousand parts water. Very large quantities must be used, — weak solutions at first to be gradually replaced by stronger and stronger ones. If its action is kept up too long the objects become brittle and are then worthless, for every section crumbles to pieces as soon as made. Chromic acid is particularly useful in studying nervous tissues, organs of sense, and other unusually delicate tissues. Its action is very slow: thus the spinal cord of a large dog or a man requires at least six weeks or two months. Chromic acid is also admirable for preparing very young and frail embryos or eggs. There are many other agents which are sometimes used for hardening, but it is not deemed appropriate to enumerate here any but the two principal and most useful ones, alcohol and chromic acid.

After the proper degree of hardness has been produced, if the piece to be cut is large enough, it may be held in one hand and cut with the other without more ado. When, however, we have to deal with something too small and delicate to be held in the hand, it is necessary to have recourse to some method of imbedding. Paraffine will usually be found the most convenient substance for this purpose, especially when mixed with one tenth of its weight of the best hog's lard. The most satisfactory process of imbedding in paraffine we have elsewhere described.[1]

On some accounts transparent soap is to be highly recommended. The best quality, containing *no glycerine*, must be chosen, then shaved into small bits, and warmed with half its volume of alcohol (as compared with it before it was cut up) until it is entirely dissolved; the specimen to be imbedded is then suspended in the warm mass by a fine thread and left for

[1] April NATURALIST, 1877, page 208.

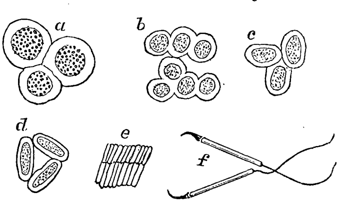
twenty-four hours. The soap does not become hard until the alcohol evaporates from it; the less alcohol, therefore, put in originally, the better. The soap ought to remain perfectly clear, enabling one to see the imbedded specimen within, so that it can easily be observed exactly in what plane every section is made, which is not possible when paraffine or wax is used. The sections, when made, if cut in soap, must be put in alcohol, if from paraffine, in spirits of turpentine, to dissolve out the remains of the imbedding mass.

If now the sections, after being thus freed from the adherent foreign matter, be mounted directly, they make poor preparations; the single parts are indistinct, and the whole is very transparent. This can be avoided by coloring them. It may be safely asserted that the introduction of staining fluids, by Gerlach, in 1858, was the most important step in advance ever yet made in histological technic. Coloring matters, as regards their action on cells, belong to two classes: either they produce a diffuse coloring of the whole cell, or they stain the nucleus much more deeply than the protoplasm and the membrane of the cell. The principal are dyes of the latter class, carmine, hæmatoxiline, and aniline blue, which are esteemed in the order named. The two former are invaluable, for by marking out the nuclei so distinctly they enable us to recognize so many centres of cells, and to observe characters which have been made prominent by their coloration, and are very different in the various forms of cells. In fact, preparations for the microscope cannot be felt or dissected, but only seen; therefore, the differential coloring produced by carmine, for example, is an assistance to the eye, comparable to the raised alphabets of the blind. In both cases, the conditions under which the special sense, whether sight or feeling, has to act are greatly exaggerated, so to speak, thus producing magnified or strengthened perceptions.

Carmine is by far the most generally useful. It is employed in various solutions, the recipes for which may be found in various hand-books, and need not, therefore, be quoted in this article. The first step in preparing it is to dissolve some of the fine-powdered carmine in a small quantity of ammonia, and it may be used at once in that form after allowing the superfluous ammonia almost entirely to evaporate. A very excellent solution may be prepared by simply adding an equal volume of rather strong acetic acid to the dissolved carmine; the exact proportion is not of very great import. Beale's carmine keeps a long time

without alteration, and Ranvier's picrocarmine has certain advantages; but on the whole, I have found the above-mentioned mixture of acetic acid and ammoniacal carmine to be quite sufficient for most work.

Hæmatoxiline, on the other hand, has to be employed in a particular solution. Dissolve first thirty-five parts of hæmatoxiline crystals in one thousand parts of absolute alcohol, and mix it cold with a solution of ten parts alum in three thousand parts distilled water. The mixture is purple at first but turns a deep blue in the course of a few weeks; but it may be used without waiting for the change of color. For use it must always be filtered through porous paper to free it from sediment, and it may be advantageously diluted with 0.5 per cent. solution of alum. It acts much more quickly and produces a deeper and more exclusive staining of the nuclei than does carmine. It is therefore particularly applicable in those cases where it is desired to study the shape and transformations of nuclei, as, for example, in tracing the development of spermatozoa. A figure is here added to show how beautifully the changes can be followed in sections of the testicle of *Epicrium glutinosum*, one of the Cœciliadæ or footless, worm-like amphibians. The testicle is divided up into numerous follicles, and the cells in each are all in one stage, while the various follicles present various degrees of development; thus in a single section all the principal alterations may be observed. The cells (Figure 71) are round at first with a very large granular nucleus (*a*). They then divide, becoming smaller and more numerous (*b*). The next change is a slightly irregular elongation of cell and its nucleus, slight at first (*c*), but gradually increasing (*d*).



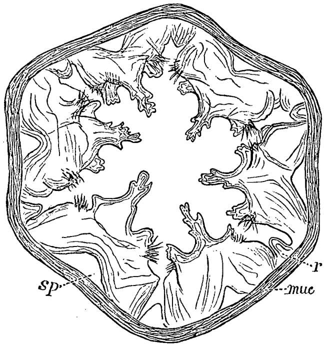
(FIG. 71.) DEVELOPMENT OF THE SPERMATOZOA OF EPICRIUM GLUTINOSUM.

At this point in the metamorphosis the protoplasm is gathered at one end of the cell, and the long nucleus at the other, and it at once becomes evident that the nucleus is to make the head of the spermatozoön, the protoplasm the tail. At this stage the cells lay themselves in rows (*e*), the nuclear ends, or as we may now call them the heads of the young spermatozoa, all pointing the same way. Each cell continues to elongate until it grows into a fully developed spermatozoön (*f*), with a pointed front end, a long head which appears almost black when stained with hæmatoxiline, and a long, fine tail. The development of the

spermatozoa seems to be very much the same in all vertebrates; that is to say, the primitive cells of the testicular follicles divide into smaller cells, and the nuclei of these make the heads, while their protoplasm changes into the tails of the spermatozoa. We have spoken of these changes here because it is proposed that the next paper shall be on the development and early stages of eggs, and there will be occasion to refer to the observations just quoted.

It is well known that cells create certain products which appear outside of the cells themselves; thus wherever there is a layer of cells having a free surface, as, for example, the outside of the body of invertebrates or the walls of tubes such as ducts of glands, the digestive canal, etc., they tend to form a structureless membrane, which stretching over them all acts as a common protective covering. The hard crust of insects is such a membrane or *cuticula*, and a corresponding one lines the tracheæ and the stomach, etc., of insects and many other animals. Now the application of section-making to the study of *cuticular* growths reveals many interesting peculiarities; as this study is only just entered upon, it is hoped that a reference to some of the results may prove valuable.

M. Léon Dufour described curiously shaped teeth in the crop of certain crickets, especially well developed in the mole-crickets, very large also in the katydids. Herr Wilde, of Leipzig, has made a very thorough study of these teeth and their development; he kindly showed the author many of his preparations, and explained his results. He made numerous beautiful sections of the crops of several species, both young and adult. Figure 72 is taken from one of his sections of the crop of *Gryllus cinereus*, the European field cricket. There are six teeth of very irregular shape, with many protuberances, but presenting, nevertheless, the general outline of a triangle, with the apex towards the middle. On each side of the projecting apex are two protruding points, at the base of which there is a bundle of stiff chitinous bristles.

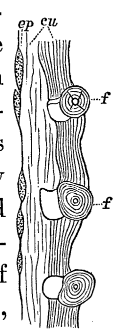


(FIG. 72.) TRANSVERSE SECTION OF THE CROP OF GRYLLUS CINEREUS.

Between every two of these gigantic teeth there is a small ridge (r) which also has a hard cuticula. Further, the teeth are not

attached along their whole base, but are partly drawn back, so that there is a space (*sp*) between the middle of the base and the muscular walls of the crop. The teeth form six regular, longitudinal rows, numbering each about twenty teeth. Their form varies according to the genera, and probably also according to the species. The walls of the crop are built up mainly of circular muscular fibres (*muc*) which by their contraction drive the teeth towards the centre and so grind up the food of the cricket, thus performing a function which we are wont to think of as properly belonging to the mouth. The study of the development of the teeth enabled Herr Wilde to ascertain that they are formed by underlying cells through a series of transformations of the cuticula, which appears at first as a simple membrane and then develops the secondary projections, which give the teeth their ultimate form. All these interesting discoveries could hardly have been made except by means of sections.

The author has himself applied section-making to the study of the tracheæ of insects.[1] It was found that the current descriptions in works on comparative anatomy and entomology were incorrect in several important particulars. The outside of the trachea is covered by a layer of flat polygonal cells, or, as it is called, a pavement epithelium. Thus in a longitudinal section of the main tracheal stem of the common water-beetle, *Hydrophilus* (Figure 73), the thin cells (*ep*) may be easily recognized by their nuclei. The epithelium secretes the enormously thick and complicated cuticula (*cu*) which makes up the rest of the tracheal wall. The well-known spiral threads or filaments *ff* are part of this cuticula, and not distinct structures as was generally supposed. These threads run around the tubes and serve as elastic supports to keep the thin walls distended; they are more or less spiral, but instead of there being but one single thread, as is usually stated, there are four or five which end, after making a few turns around the tracheæ, new ones arising to replace them. As the fibres run transversely, of course their cut ends only are seen in a longitudinal section like Figure 73. But these ends show that the filaments consist of a lighter outside, and a darker inside portion, which latter is round. The rest of the cuticula (*cu*) is divided into two layers,



(FIG. 73.) LONGITUDINAL SECTION OF THE TRACHEA OF HYDROPHILUS PICEUS.

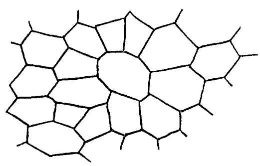
[1] Minot, Recherches histologiques sur les Trachées de l'Hydrophilus piceus. Arch. de Physiol. normale et pathologique, sér. ii., tom. iii., page 1.

the inside or right hand one in the cut, being slightly colored by carmine, while the outside layer is hardly stained at all. This affords another excellent illustration of the ease with which valuable discoveries may be made, when well-known histological methods are applied to the study of insects ; indeed, insects offer a rich and easily accessible field of research, promising perhaps greater rewards in proportion to the necessary labor than almost any other department of zoölogical investigation.

It would be easy to add illustration after illustration to those already given, but it is not our purpose to review the progress of histology, but merely to give incentives to work in that field. We pass on, therefore, to a few additional considerations on the "technique" of preparing tissues for microscopical examination. Experience has shown that it is very difficult to distinguish the single cells in sections, in some case almost or quite impossible; or it is even impossible occasionally to make any sections at all. On these accounts various means are employed either to isolate a few cells or to mark the outlines of them. The methods hitherto employed for these purposes are few in number and limited in application, but they have already led to interesting observations.

Many cavities of the body, both of vertebrates and lower animals, are lined by a layer of flat cells that are separated by lines of intercellular substance; by treating such a surface suitably with certain silver salts the intercellular lines are colored dark brown or black. A solution of one part of nitrate of silver in five hundred parts of distilled water (by weight) is very convenient. It gives beautiful preparations when applied to the mesentery of a rabbit, for example. The mesentery is the thin membrane by which the intestine is suspended from the back of the abdomen. Cut out a small piece from a freshly killed animal, a frog or rabbit or any other vertebrate, and place it in a silver solution, where the direct rays of the sun can fall upon it, and move it about with a glass rod (metal would be corroded) so that all parts may be equally acted upon ; next remove it for a moment into distilled water to wash off the silver, and then spread it out on a glass slide and let it dry almost completely, taking great pains to stretch it out by pulling it at various points so that it shall dry *fully* extended. Before it is quite dry put on a drop of glycerine and a thin glass cover in the usual way. If the impregnation has been successful, the lines will appear very sharply, as in Figure 74, which is from the mes-

entery of a turtle. If the impregnation was not sufficient the lines do not appear, but that is also the case if it has been too prolonged, for then the cells fall off altogether. The membrane may be colored with hæmatoxiline or carmine, if so desired, after impregnation, and then the stained nuclei appear within the dark outlines making exceedingly pretty preparations.



(FIG. 74.) MESENTERY OF TURTLE. SURFACE IMPREGNATED WITH SILVER.

Maceration gives the means of isolating layers of cells. If the skin of an amphibian, a toad, for example, be pinned out on a bit of cork and then placed in a dish of water containing three or four drops of strong carbolic acid to prevent the development of germs, and then left for a day or two, the superficial layer of cells may be peeled off with a pair of pincers, and so on, successive layers from day to day until the whole skin has been removed. The bits thus peeled off usually contain but a single layer of cells, and if colored with carmine they make very beautiful preparations.

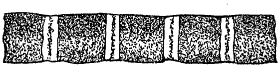
But besides investigating cells in their relation to one another, the histologist endeavors to determine the form of single cells, and employs therefor means of isolation or dissociation. These may be either mechanical, such as shaking up a tissue in a fluid or teasing it out with fine needles, etc., or chemical. Usually a combination of the two is the most effectual.

In most tissues the cells are united by intercellular matter, just as above described in the epithelium of the mesentery. This substance acts as a cement binding the cells together. In some cases it reaches an extraordinary development, so that the cells come to be quite far apart, as in cartilage, for instance. But usually it is very thin, and may be dissolved, in some cases, without altering the appearance of the neighboring cells. The cells that line the intestine and stomach are particularly adapted to illustrate this action of certain chemicals. Thus if a small bit of the wall of the digestive canal be left in alcohol of thirty per cent. for twenty-four hours, the lining cells all become loosened so that they are easily scraped off with a needle or scalpel, and if mounted in glycerine mixed with a little picrocarmine, they become stained in a week or so, and show the details of structure of the single cells very admirably.

Chromic acid has a similar action, and solutions of two parts in ten thousand of distilled water have a great value from their so

affecting the brain that the ganglion cells may be quite easily isolated. To effect this a very small piece of the brain — calf's brain is perhaps the best — is placed in fifty or sixty times its volume of the solution for twenty-four hours, and then carefully teased out under a good dissecting microscope.

Both weak chromic acid and alcohol may be used for isolating muscular fibres. Flies and beetles are perhaps the best for this purpose. The muscles of the wings (not those of the legs) should be torn out with fine forceps, and little bits, the smaller the better, placed in thirty per cent. spirit for twenty-four hours, and then dissociated or pulled apart on a glass slide, with fine needles. With sufficient care it is possible to separate the single fibrillæ of each fibre, and when stained with hæmatoxiline the alternating lines, dark and light (Figure 75), appear very sharply. These lines are those that make the muscles transversely striated. The cause of this striated appearance is not yet fully determined, but it is apparently connected with greater perfection of the muscular fibre than is found in the unstriated form. Different as is muscle in appearance from cells yet it originates from them, and is in fact formed of metamorphosed cells, by a series of changes all as great as those which produce bone.


(FIG. 75.) ISOLATED MUSCULAR FIBRE OF COMMON WATER BEETLE.

We have still to notice a very important class of procedures, namely, injections. In the higher animals we find two distinct sets of vessels ramifying through the whole body: one of these is the system of blood-vessels, the other the lymphatic system. As is well known to all, the blood-vessels branch out into very fine tubes that form a complicated net-work in every part of the body, so fine that it can only be followed when the tubes or capillaries have been artificially filled with a colored matter. The same is true of the lymph-vessels, but to an even greater extent. Many of the structures of the body are permeated by connective tissue, and in this tissue there are numerous cavities filled with fluid; they are in communication with very delicate tubes, the lymphatic capillaries, which soon unite into larger canals, and these form branches which gradually join together and lead to the thoracic duct or main stem, which empties into the veins just before they open into the heart. The branches of this tubular system are provided with valves so arranged that the liquid contained in the tubes can only pass upward or towards the main stem. Now when any

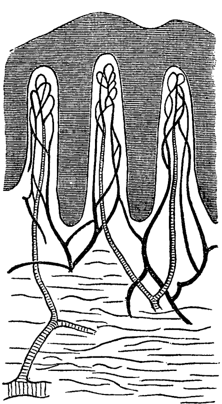
motion takes place, some of the liquid in the cavities of the connective tissue is pressed into the lymphatics and so slowly driven onwards into the heart. To counterbalance this loss of intercellular fluid, certain constituents of the blood exude through the walls of the capillaries and keep up the supply. There is, therefore, a double circulation: one within the blood-vessels, and another from the walls of the capillaries through the lymphatics. The liquid in both circulations is ultimately returned to the heart.

Different methods have to be employed for injecting the two systems. In the case of the blood-vessels a rather large syringe may be used, provided with a point small enough to pass into the artery of the part to be injected. The artery should be carefully laid bare and cut about half way through; the point of the syringe, which must be previously filled with the injection mass, is pushed into the artery and firmly tied in place. In many cases it is quite sufficient to inject a cold saturated solution of Prussian blue in water, or when more perfect preparations are wanted, a little gelatine may be added; in this case, however, there arises the inconvenience that both the injection mass and the organ to be injected have to be kept warm while the operation is going on, otherwise the gelatine solidifies.

To prepare a "warm" injection mass, the following method is, perhaps, the best. A solution of Prussian blue is necessary; this the histologist must make for himself. To do this take a concentrated solution of sulphate of protoxide of iron in distilled water, and pour it slowly into a concentrated solution of yellow prussiate of potassium; a precipitate of insoluble Prussian blue is formed. There should be a small excess of prussiate at the end of the operation, to test which take out a drop and add to it a little of the sulphate. If there is any free prussiate still present, a blue precipitate is thrown down. Filter through a felt strainer, below which a funnel with a paper filter has been placed. Pour water on to the strainer in small quantities at a time, and continue filtering; this operation must be kept up for several days, until the liquid below the second filter appears distinctly blue. The matter on the felt strainer is then removed and dissolved in distilled water. This solution is admirable for cold injections or for filling the lymph-vessels, as will be described presently. There should always remain an excess of blue in the vessel in order to be sure that the solution is saturated; as the solution is removed it may be replaced by dis-

tilled water, as long as there is any blue left. To make the "warm" injection mass take twenty-five parts of the Prussian-blue solution and one part gelatine. The latter must be of the finest quality, as otherwise it produces a granular precipitate which renders it useless for histological purposes. Put the gelatine to soak for half an hour in distilled water, then remove and wash it; place it in a glass vessel and warm it in a water-bath, when it will melt in the water it has absorbed. The Prussian blue is put in another vessel in the same water-bath, so that the two liquids are at the same temperature. Pour the gelatine, little by little, into the blue, stirring constantly with a glass rod. Keep on warming and stirring until the granular precipitate formed at first disappears. Upon being filtered through a piece of clean flannel, the mass is ready for use.

It requires only to be slightly warmed to become liquid, and the organ to be injected does not need to be heated to so high a temperature as is necessary in using many other injection masses; there is, therefore, no danger of injuring the tissues by subjecting them to too high a temperature. The injection should be continued until a little while after the mass begins to come out through the veins, in order to allow all the capillaries time to fill themselves. When the injection is finished, the organ may be placed to advantage for twenty-four hours in a 2 to 1000 solution of bichromate of potassium in distilled water, and then be removed to alcohol; or it may be put at once in alcohol, and, when hardened, sections made of it. The sections should be pretty thick, and may or may not be stained as is desired. If too thin, they do not show the connections of the vessels.



(FIG. 76.) INJECTION OF HUMAN LIP.

As an example of the clearness with which the blood-vessels may be traced in a successful preparation, a figure of a section through an injected human lip is given (Figure 76). The shaded portion represents the skin proper, and is penetrated by papillæ sent up from the underlying connective tissue, known in anatomy as the cutis, and carrying the blood-vessels. There is a net-work of small arteries in the cutis, and from this there pass up from three to five fine branches into each papilla, and form by division and inter-communication a wide capillary net-work. One or several fine capillaries bend

round and form the veinlet which passes down the middle of the papilla, from top to bottom, in a nearly straight line, and sometimes taking up fine branches on the way until it finally connects with the venous net-work of the cutis.

This arrangement of the vessels is very characteristic; similar ones occur elsewhere, where there are well-developed papillæ, as, for instance, on the tongue or in the intestine. But each organ presents characteristic peculiarities in the distribution of its blood-vessels, and to an experienced histologist the veins, capillaries, and arteries of the liver and kidney, etc., are as distinctive of each organ as is its general shape and appearance.

As the presence of the valves does not permit us to inject the lymphatics from a large stem in the finer branches, as in the blood-vessels, a different method of forcing in the fluid has to be adopted. A small syringe with a very fine sharp point, such as is known among instrument-makers as a hypodermic syringe, must be used. The point is made to penetrate in the connective tissue, and the colored liquid — the best is a solution of Prussian blue — is forced out slowly and gently, and fills at first the cavities of the tissue and then the small lymphatics. These injections are difficult to make and by no means always succeed well. Perhaps the best place to try first is the interdigital web of the hind-foot of a frog, or the outer half, that is, the muscular part of the walls of the small intestine; but the easiest of all to fill are the lymphatics of the dog's testicles. When the injection has been once made in the way indicated, the tissue or organ may be hardened for cutting either in chromic acid or in alcohol.

Such, then, are some of the principal means employed to investigate the microscopical structure of animals. They all have this much in common, that they are endeavors to render certain characters more visible than they are naturally. This we do whether we stain the nucleus, or inject the blood-vessels, or isolate single cells. It may well be added that a good knowledge of optics is necessary to a good histologist.

The worker should also remember that American instruments are usually much less convenient and practical than the German and French microscopes, while the lenses are no better, though enormously more expensive. The writer personally likes Zeiss's instruments very much. As this optician manufactures his objectives upon mathematical principles, he is able to make them all nearly alike; but it must be understood that there are many others whose objectives are also of the best quality. At present

there is no difficulty in getting the best lenses and instruments, providing an American or English microscope of large size and complicated structure is not chosen. It will be found that those only who use a microscope for amusement utterly condemn the simple instruments, while those who make investigations and gather wide experience often assert that the greater the simplicity the better. The European histologists I have met generally use a stand without rack and pinion for coarse adjustment, without movable stage and without movement round a horizontal axis.

As to books, Frey's Manual, of which there has been a translation published in New York, is only pretty good. It came into general use because it was for a long time without rivals. There have lately appeared two little works on this subject, in England, one by Professor Rutherford, the other by Mr. Schaeffer, both of which are considered good. But by far the most important work is Ranvier's Traité Technique d'Histologie now being published in Paris, in numbers, three of which have already appeared. The moderate price of the book, — only twenty-five francs for a volume of a thousand pages, — the fullness of detail, and the superb illustrations alone are sufficient to recommend the work. M. Ranvier has written a treatise which will probably always be remembered as one of the most important and valuable manuals ever published, and which ought to be owned by every one who attempts to investigate the elementary structure of animals.

CONCERNING FOOT-PRINTS.

BY I. C. RUSSELL.

ICHNOLOGY (foot-print lore) is the name which has been applied to one of the most attractive and interesting paths of research that geology has pointed out. This branch of palæontology[1] has for its object the study and interpretation of the many fossil foot-prints that have been found in the rocks, which were impressed there by the feet of animals when the material of which those rocks are composed was the shifting sands along some ancient shore. The study of foot-prints has at length been recognized as a distinct and important branch of palæontology, one which has often afforded the only means for judging of the character and structure of the ancient animals that have left no other records of their existence than the impressions of their feet.

[1] From palaios, *ancient;* onta, *beings;* logos, *a discourse.*